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**IMPROVING SOIL PROPERTIES WITH *ACACIA SEYAL* AGROFORESTRY AND BIOCHAR:
IMPLICATIONS FOR SORGHUM PRODUCTION ON THE DRYLANDS OF SOUTH SUDAN**

DOCTORAL THESIS
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ACADEMIC DISSERTATION

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ABSTRACT

Rainfed agriculture is a vital land use practice for food security and economic development in most of drylands, but particularly in sub-Saharan Africa (SSA). However, it is becoming an increasingly uncertain and inefficient practice in SSA because of climate change and extremes (i.e. low and erratic rainfall, high temperatures, floods, and drought occurrence), and low soil fertility and water supply. For example, yields of sorghum, which is the main staple food crop in South Sudan, are dwindling under rainfed cultivation in its main production areas in the north of the country due to the previously mentioned factors. Nevertheless, soil amendment materials, such as biochars, along with integration of sorghum production into agroforestry systems, which can improve soil fertility and water storage capacity, could assist in improving the crop yields.

In this dissertation, the effects of *Acacia seyal*-based agroforestry and addition of biochar on soil water retention and supply and on sorghum yields were examined. The research focused on 1) the potential of using biochar as a soil amendment combined with *A. seyal*-based agroforestry in a field experiment, 2) the effect of biochar on alleviating water stress on sorghum yield in greenhouse conditions, and 3) simulation of the potential effect of biochar amendments on improving sorghum biomass and grain yield, especially as indicated by differences in yield between wet and dry years.

The two-year agroforestry field experiment (Paper I) was carried out at Magara Village north of Renk in South Sudan, during the growing seasons of 2011 and 2012. The split block experiment included three *A. seyal* tree density treatments: no trees; scattered trees (100 trees ha⁻¹) and dense trees (400 trees ha⁻¹) and two biochar amendment treatments (0 t ha⁻¹ and 10 t ha⁻¹). The soil consisted of silty loam underlain by clay, and the biochar source was *A. seyal* trees. A soil analysis showed that agroforestry resulted in lower soil pH, N, and total and exchangeable Ca²⁺ contents and higher C/N ratios compared to sole sorghum cultivation. The application of biochar significantly increased the soil C and exchangeable K⁺ contents as well as the pedotransfer-derived field capacity and plant available water contents, but significantly decreased the content of exchangeable Ca²⁺ and cation exchange capacity. The inclusion of *A. seyal* trees significantly decreased the sorghum grain yields, and the effect of biochar on grain yield compared to sole sorghum cultivation without amendment was not significant. The Land Equivalent Ratio (LER, the sum of the fractions of the intercropped yields divided by the sole-crop yields) value was 0.3 for dense *A. seyal* intercropping combined with biochar in both 2011 and 2012 and with scattered *A. seyal* intercropping in 2011, but it was twofold greater (0.6) in 2012 with biochar amendment.

The greenhouse experiment (Paper II) was carried out at the Viikki Campus, Helsinki, Finland, during May–December 2011. The main factor was drought stress with three levels of soil moisture content: 60% of field capacity (well-watered), 40% (medium drought) and 20% (severe drought). The same type *A. seyal* biochar, in the same amounts as applied in the field experiment (0 t ha⁻¹ and 10 t ha⁻¹), was used. Drought stress had a significant effect on sorghum gas exchange but not on sorghum stomatal traits. The stomatal conductance and photosynthesis and transpiration rates were all significantly reduced under severe drought compared to values found in plants that were under medium drought or well-watered. The photosynthetic water use efficiency (WUE) increased with the level of drought stress. Drought stress significantly reduced the sorghum biomass and grain yields compared to those observed in well-watered plants. Biochar addition did not have a significant effect on any of sorghum stomatal traits, gas exchange or grain yield.

The biochar/sorghum simulation study (Paper III) was carried out using the water-driven crop-growth model AquaCrop (version 6.1). The model was parameterized for the field experiment site and soil conditions. Soil fertility stress parameters were adjusted so that simulated biomass and grain yield values best matched the levels recorded in the field experiment. Climate data for 2011 and 2012, both wet years, and for 1990, an extremely dry year, were extracted from the Climate Forecast System Reanalysis (CFRS) online dataset. The effects of biochar were simulated using the changes in soil hydraulic properties (increases in field capacity, available water capacity and saturated conductivity) reported in a published meta-analysis study. Generally, the simulated biochar amendments having the greatest effect on soil hydraulic properties increased the water content of the rooting depth in all three years, but an increase in sorghum production was only discernible for 1990.

The results from paper I showed that sorghum yields are lowered when the crop is grown in agroforestry systems. As sorghum is not tolerant of shade, the reduction in sorghum production with increasing tree density was probably due to canopy cover and shading. This effect thus overrode any benefit of having the trees in the cultivation system. The results from paper II indicated that biochar has no significant effect on alleviating drought stress on sorghum production and grain yields, while the results from paper III showed biochar, while improving soil hydraulic properties, only resulted in increased sorghum biomass production and grain yield in very dry years.

Overall, the results from this study showed that the propounded benefits of agroforestry and biochar need further study and critical assessment, particularly in semi-arid environments where the water supply through rainfall is low and erratic but the water demand is constantly high. The results may well vary with different crops and agroforestry systems, as well as with different soil types and the type and dose of biochar. Furthermore, the results may only become apparent with sufficient time and therefore long-term studies are needed.

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LIST OF ORIGINAL PUBLICATIONS

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AUTHORS’ CONTRIBUTIONS

Contribution of authors to the original articles of this dissertation is displayed in the following table:

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Planning the study	OL, JH, BD	JH, BB, BD, OL	MS, JH, BD
Data collection	BD, PT	BB	BD, MS
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LIST OF ABBREVIATIONS

AGB	Aboveground plant biomass
ANOVA	Analysis of variance
AWC	Plant-available water content
BC	Biochar
C	Carbon
CC	Canopy cover
CCo	Initial canopy cover
CGC	Canopy growth coefficient
CN	Curve number
C/N	Carbon-to-nitrogen ratio
CEC	Cation exchange capacity
CO ₂	Carbon dioxide
DBH	Diameter at breast height
DM	Dry matter
DOY	Day of year
ET	Evapotranspiration
GDD	Growing Degree-Days
ET _o	Reference evapotranspiration
FC	Field Capacity
GHG	Greenhouse gases
HI	Harvest index
HI _o	Harvest index for crop grown under optimum conditions
H ₂ O	Water
ICARDA	International Center for Agricultural Research on Dry Areas
ICP-OES	Inductively coupled plasma - optical emission spectrometry
ICRAF	International Centre for Research in Agroforestry
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
K _{sat}	Saturated hydraulic conductivity
K _s	Stress coefficients
LAI	Leaf area index
LER	Land Equivalent Ratio
MAP	Mean Annual Precipitation
N	Nitrogen
PAH	Polycyclic aromatic hydrocarbons
PWP	Permanent wilting point
SEM	Scanning electron microscopy
SOC	Soil organic carbon
SOM	Soil organic matter
SSA	Specific surface area
SSA	sub-Saharan Africa
T _{base}	Default base temperature
T _{upper}	Default upper temperature
Tr	Transpiration
v/v	Volume per volume
w/w	Weight per weight
WP	Water productivity
WP*	Normalized water productivity
W _r	Rooting zone
WUE	Water Use Efficiency
θ _{FC}	Volumetric water content at field capacity
θ _{PWP}	Volumetric water content at permanent wilting point
θ _{SAT}	Volumetric water content at saturation

IMPORTANT DEFINITIONS

Agroforestry:

Agroforestry systems are forms of land use systems where woody perennials (trees, shrubs, palms etc.) are deliberately used on the same land management as agricultural crops and/or animals, in some form of spatial arrangement and temporal sequence (FAO 2015).

Aridity index

Aridity index is the ratio of mean annual precipitation to mean annual potential evapotranspiration (UNEP 1993)

Biochar

Biochar is a carbonaceous porous solid material, produced by thermochemical conversion of biomass in anaerobic conditions (pyrolysis), that has physiochemical properties suitable for the safe and long-term storage of carbon (C) in the environment and, possibly, soil improvement (Shackley and Sohi 2010).

Model

A model is a “schematic representation of the conception of a system or an act of mimicry or a set of equations, which represents the behaviour of a system” (Murthy 2002). A model can also be defined as “a representation of an object, system or idea in some form other than that of the entity itself” (Murthy 2002).

Drylands

Drylands are broadly defined as “land areas with an aridity index (ratio of mean annual precipitation to mean annual potential evapotranspiration) of less than 0.65 (UNCCD 1994)

Land equivalent ratio (LER)

The land equivalent ratio (LER), the sum of the fractions of the intercropped yields divided by the sole-crop yields, is used to judge the effectiveness of intercropping systems (Mead and Willey 1980). A value of 1.0 is the critical value, above which intercropping is favoured and below which monocropping is favoured.

Soil amendment

Soil amendments are both materials and practices used to improve soil quality in terms of its structure and biochemical function.

1 INTRODUCTION

1.1 Improving crop productivity in sub-Saharan Africa: needs and methods

Drylands are distinguished by low precipitation and/or alternatively an aridity index (ratio of mean annual precipitation to mean annual potential evapotranspiration) of < 0.65 (UNCCD 1994; Safriel et al. 2005). They cover approximately 40% of the world's land area and support two billion people, 90% of which live in low income countries (Reynolds et al. 2007; UNEP-GEO 2007). Drylands in Africa are estimated to cover about 43% of the land surface, but constitute about 75% of the agricultural land (Raffaello and Morris 2016). Dryland ecosystems are vulnerable to climate change extremes, especially rainfall variability, droughts, floods and heat waves (Thornton et al. 2011, 2014). This constitutes a great threat to rainfed agriculture, which is practiced in many dryland regions and the major source of livelihoods in SSA, putting food security at risk (UNEP-WCMC 2012).

Besides climate, there are other factors that affect agricultural production in dryland, including poor soil fertility, lack of agricultural inputs (e.g. agrochemicals and agricultural machinery) and poor agronomic practices (Humphreys et al. 2008). Soil fertility is of particular importance in SSA agriculture as it is not only a cause of dwindling per capita food production but also the reason behind the poverty trap as smallholder farmers are poor and cannot afford fertilizers resulting in low crop yields (Morris et al. 2007; Twomlow et al. 2008; Lal 2009; Barrett and Bevis 2015). Food security is therefore of great concern in SSA as the population is expected to increase two and half fold and the demand for cereals to triple by 2050 (van Ittersum et al. 2016).

Land degradation (low SOC and soil nutrient imbalance) is a common constraint for crop production not only in SSA but throughout the tropics (Lal 2015). Millions of hectares of productive lands in Africa are lost annually because of land degradation (Bationo et al. 2006). Although soil fertility management efforts in Africa have a long history and have had significant influence on agricultural production in other parts of the world, there has been little achievement in Africa (Lal 2015). The use of agrochemicals, improved seeds and irrigation, which was the driver of the boom in agricultural production during the Green Revolution of the 1960s and 70s, has had limited effects on agricultural production in Africa (Smaling 1993).

Soil fertility management strategies are currently based around the concept of the integrated soil fertility management, which aim to combine all site-specific processes (i.e. biological, physicochemical, socio-economic, health, nutrition and political) that influence soil fertility (Bationo et al. 2006). Soil fertility interventions to address soil degradation and low agricultural productivity in SSA need to be site specific and effective in compensating for soil nutrients loss through crop mining, water supply, runoff, erosion, and promote better ecosystem services.

A number of international organizations, e.g. International Center for Agricultural Research on Dry Areas (ICARDA), International Centre for Research in Agroforestry (ICRAF) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), have exerted great efforts to improve agricultural production in drylands (FAO 1997). ICRAF has pioneered efforts to incorporate trees into cropping systems and to use of climate smart production methods to create resilient cropping systems and long-term improvements in crop yields (Nair and Garrity 2012). Adoption of agroforestry, such as parkland systems, in semi-arid Africa in which *Faidherbia albida*, Shea (*Vitellaria paradoxa*), and Gum acacia (*Acacia senegal*) are used have been reported to improve both soils and crop yields (Boffa 1999; Luedeling 2012; Abdoukadi et al. 2019; Ereso 2019).

In general, attempts to raise crop yield through soil amendments that increase soil organic matter contents (i.e. trees on farms and addition of organic materials) has a long history in SSA (Bationo et al. 2006). Although often low in nutrients, soil moisture stress is often the main problem of dryland agriculture, and its impact on crop production is increasing in SSA due to climate extremes (Blanc 2012). However, soil moisture stress is a function of both low and erratic precipitation, and the ability of the soil to hold and release moisture (Bationo et al. 2006). Therefore, the use of soil amendments to improve soil water retention and supply to the crops may offer means to mitigate against drought and water stress, restore degraded soils and improve crop yields.

1.2 Agroforestry

Agroforestry is a form of land use systems where woody perennials (trees, shrubs, palms etc.) are deliberately used on the same land management as agricultural crops and/or animals in some form of spatial arrangement and/or temporal sequence (FAO 2015). Agroforestry is increasingly being recognized as a potential land use strategy to improve food security in low and middle-income countries, besides its environmental and socio-economic benefits, including combating climate change (Waldron et al. 2017; Brown et al. 2018). The benefits of agroforestry are also recognized in high-income countries, especially in marginal areas where soil fertility is poor and climate and topography constrain intensive agriculture (Plieninger et al. 2015). The benefits of agroforestry in SSA would be particularly valuable because of the long history of land and soil degradation and continuous declines in per capita farm income (Verchot et al. 2007; Mbow et al. 2014; Barrett and Bevis 2015; Bayala et al. 2018).

Besides the inherent benefits of the trees (organic matter accumulation via pruning, litterfall and root decay; increasing water infiltration; reducing soil evaporation and surface runoff; nutrient recycling; carbon sequestration; and soil stabilization), they can provide a wide range of goods and services, including food, shade, fodder and non-timber wood products (e.g. gums and resins, fuelwood and charcoal, medicinal and other products) (Idol et al. 2011; Nair and Garrity 2012; Bayala et al. 2014). However, there are different accounts of the benefits of agroforestry on crop production, particularly in terms of whether the trees support (facilitate) field crop production or compete with them for light, water and nutrients. Thus, while some agroforestry studies on drylands have reported facilitation effects of trees (positive interactions) on crop yields, which were manifested in higher Land Equivalent Ratio (LER) values (> 1.0) (Young 1990; Nair 1993; Palm 1995; Ong and Leakey 1999; Hauggaard-Nielsen and Jensen 2005; Fahmi 2017), other studies have found competitive interactions between trees and crops resulting in lower LER values (< 1.0) (Kessler 1992; Suresh and Rao 1999; Nair and Garrity 2012; Ndoli et al. 2017). Other studies have found facilitation or competition effects depending on the development stage of both crop and tree, amount of rainfall, woody plant used, and soil (Ludwig et al. 2004; Dohn et al. 2013; Blaser et al. 2013; Priyadarshini et al. 2016; Muler et al. 2018).

Despite the success of agroforestry systems in many world regions (Zhaohua et al. 1991; Rao et al. 1998; Brown et al. 2018), their adoption has not yet been fully realized in Africa. This is due to various reasons, such as lack of political support, complexity of agroforestry systems and lack of skills and relevant technologies, and population growth (Mbow et al. 2014; Kiyani et al. 2017). In addition, land or tree tenure issues have limited the adoption of agroforestry systems, especially in countries where they are based on naturally regenerated tree species (e.g. *Acacia senegal* and *Acacia seyal*) as in Sudan (Raddad et al. 2007). Glover (2005) thus highlighted the importance of land tenure and ownership devolution from the state to communities and households in Gedaref state, Sudan. Unless there is land-use policy reform that recognizes the farmers' land and tree use rights, it is difficult to get farmers to invest in agroforestry systems in spite of the socio-economic and food production benefits (Fahmi et al. 2017).

1.3 Biochars in semi-arid agriculture

Biochars are solid pyrogenic carbon materials produced by thermal decomposition of biomass under limited or absent oxygen and used as soil amendment to increase fertility or sequester atmospheric (Mukherjee et al. 2011). The historical reference of biochars to the fertile Amazonian dark soils (Terra Preta) in Brazil had inspired researchers and scientists from different sectors about potential benefits of biochars (Smith 1980; Glaser et al. 2002; Lehmann et al. 2003). Furthermore, the use of biochar in agriculture is increasingly still attracting attention globally since the past decade due to their agronomic and environmental merits (Hussain et al. 2016; Obia et al. 2019). The past research that focused on effects of biochars on soil fertility have revealed significant improvements in water retention (Bruun et al. 2014; Scholz et al. 2014; Lehmann and Joseph 2015; Omondi et al. 2016; Fischer et al. 2018), and soil structure (Glaser et al. 2002; Bruun et al. 2014; Obia et al. 2016; Obia et al. 2017) after addition of biochars.

Some biochars are reported to induce significant improvements in chemical properties of some soils such as increase in cation-exchange capacity CEC (Jien and Wang 2013) and pH (Xu et al. 2013), and decrease in acidity due to liming effect (Xu et al. 2013). In addition, biochars can act as a sources for nutrient depending on the richness of their feedstocks (Laird et al. 2010; Major et al. 2010a; Xu et al. 2013), and increase nutrient retention as well due to changes in CEC, soil structure, and improved fertilizer efficiency (Steiner et al. 2007;

Laird et al. 2010; Major et al. 2010a; Biederman and Harpole 2013; Liu et al. 2013; Martinsen et al. 2014; Scott et al. 2014). Addition of biochars to soil is reported to improve soil microbial activity through habitat improvement (available water and nutrients).

Generally, improvements in soil fertility by biochars are more evident in coarse textured soils than the fine textured ones because of soil hydrological improvement and nutrient retention (Blanco-Canqui 2017; Bohara et al. 2019). On the other hand, the effects of biochars on crop growth and yields vary greatly, and crop yields are influenced by prevailing climate, soil, crop and biochar types (Jeffery et al. 2011; Biederman and Harpole 2013; Liu et al. 2013). For instance, Jeffery et al. (2017) have reported from their meta-analysis study an increase in crop yields by 25% (on average) in tropics due to biochars addition, whilst, crop yield increase was smaller or negative in temperate regions.

Although there is meagre biochars research published from Africa, several studies had reported an increase in yield of cereal crops such as maize after biochars addition (Kimetu et al. 2008; Cornelissen et al. 2013; Martinsen et al. 2014; Yeboah et al. 2016; Mensah et al. 2018; Calys-Tagoe et al. 2019; Kätterer et al. 2019). However, specific studies dealing with effects of biochars on sorghum growth and yields are scarce (Mohamed and Hammam 2019; Oziegbe et al. 2019).

The effects of biochars application on agroforestry systems specifically on soil and crop yield improvements are not sufficiently researched; however, the literature is rapidly growing. Stavi (2013) has highlighted the importance of use of biochar in forestry, agroforestry and other tree based ecosystems as a tool for climate change mitigation and adaptation. The same author has noted that the studied forest systems responses were positive to charcoal or ash from the wild fire, rather than the specially produced biochars. In their review study, Stavi and Lal (2013) also have reported the importance of combined agroforestry and soil addition of biochars in agroecosystems to increase agronomic productivity, support ecosystem services and sequester carbon in the long-term.

Recently, Miltner and Coomes (2015) have reported a successful addition of biochar into swidden-fallow agroforestry systems in Iquitos, Amazonian Peru in what they termed as kiln site agriculture. Charcoal kilns were set in the fallow sites to support the poor farmers besides growing, annual, and perennial crops such as manioc (Cassava) which is their main staple crop. In addition, farmers used kiln soil and biochar off-site, in home gardens, nursery beds, and planting holes (Coomes and Miltner 2017).

1.4 Sorghum

Sorghum (*Sorghum bicolor* L. Moench) belongs to the family of Poaceae and is the fifth most important cereal crop in the world after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.). Sorghum is believed to have originated in Sudan and Ethiopia in East Africa some 3000 years ago (FAO 1995). It is a multipurpose crop that provides food, feed, fiber and biofuel (Zheng et al. 2011). Sorghum is currently cultivated in all continents and tropical, subtropical and temperate regions (Popescu et al. 2014), but it is well adapted to semi-arid tropical environments because it is more tolerant of drought and high temperatures than other major cereal crops (e.g. maize, rice, wheat and barley) (Ati and Akinyemi 2018). It is the main food crop throughout Africa, Central America and South Asia (FAO 1995; Ati and Akinyemi 2018). World sorghum production and consumption in 2014 was estimated at 63,811 and 63,148 thousand metric tons respectively (Popescu et al. 2014). Besides being a staple food, sorghum is recently being used for biofuel production (Mathur et al. 2017). Although sorghum production has enormous potential to increase globally, the crop yields are below the potential yields due to poor soil, cultivation and variety (Mundia 2019). Sorghum yields are even lower and variable in the subsistence rainfed farming systems of the SSA (Msongaleli, et al. 2017).

Increasing recurrence of droughts are negatively affecting agricultural production worldwide (Dimkpa et al. 2019), but the effects are more devastating in arid and semi-arid regions (Badigannavar et al. 2018). Although sorghum is drought tolerant, it is susceptible to drought at certain growth stages, particularly at reproductive stage (Batista et al. 2019).

Sorghum yields when intercropped with acacia trees in arid and semi-arid regions have been variable depending on tree densities, age and agricultural practices (Bayala et al. 2014). For example, intercropping sorghum with *Acacia saligna* (Droppelmann et al. 2000) and *A. senegal* (Fadl 2013) significantly increased

sorghum biomass and grain yields due to facilitation effect. In contrast, Suresh and Rao (1999) and Gaafar et al. (2006) observed that intercropping sorghum with *Faidherbia albida*, *Acacia ferruginea*, *Albizia lebbek* and *Acacia senegal* significantly reduced sorghum yields due to competition between trees and crops over resources.

The effects of soil amendment with biochar on sorghum production in arid and semi-arid regions has not been widely reported. Blackwell et al. (2015) and Laghari et al. (2015; 2016), however, have reported increase in sorghum plants growth and yields. Blackwell et al. (2015) have attributed benefits of biochars to sorghum yields to improvement in mycorrhizal colonization and nutrient availability in a sandy soil with low nutrient in Australia. Laghari et al. (2015, 2016) have explained that the improvement witnessed in growth of sorghum plants and related yield increase when biochars were added to the Chinese sandy desert soils was a resultant of improved soil hydraulic and chemical properties.

1.5 Agriculture in South Sudan

The Republic of South Sudan is the world's newest country created after its independence from Sudan on 9 July 2011. The country is landlocked and lies in the Central East Africa between latitude 3.5° and 12° North and longitude 24° to 36° East. It shares borders with Sudan in the north, Ethiopia in the east, Kenya, Uganda and the Democratic Republic of Congo in the south, and Central African Republic in the west. The total land area is approximately 620,000 km², and it has a population of 12.6 million. The dominant climate in South Sudan is a tropical savanna climate (Aw), but arid (MWh) and semi-arid (BSH) climates are predominant in the north of the country and in southeastern parts of Eastern Equatoria (Köppen 2016; NAPA 2016). Mean annual temperature ranges between 26 and 32°C, and the annual rainfall varies across the country between 200 and 1500 mm. The duration of the growing season varies between 100 and 250 days. While most of the country has two cropping seasons (April-June and July-December), the arid and semi-arid areas have only one cropping season (July-October).

Agriculture in South Sudan is rainfed and subsistence based but has huge potential if wisely developed and utilized (Diao 2012). Although only 5% of the country's total arable land area (30%) is utilized for crop production, agriculture is the main livelihood activity for rural populations (MAFC and RD 2012). However, the different soil types and climatic conditions offer opportunities to cultivate various food and cash crops. The main staple crops in South Sudan are sorghum, maize (*Zea mays* (L.)), cassava (*Manihot esculenta* (Crantz)), rice (*Oryza sativa* L.) and pearl millet (*Pennisetum glaucum* (L.) R.Br.). Cash crops are groundnut (*Arachis hypogea* L.), sesame (*Sesamum indicum* L.), pulses, cotton (*Gossypium barbadense* L.) and recently sunflower (*Helianthus annuus* L.) (MAFC and RD 2012). In addition, there are small-scale plantations of coffee (*Coffea arabica* L.), tea (*Camellia sinensis* (L.) Kuntze), sugarcane (*Saccharum officinarum* L.) and tobacco (*Nicotiana tabacum* L.).

Forests make up to 23% of the country's total land cover and are important national assets and a valuable livelihood source. The forests are classified to closed forests, open forests and woodland, and savanna with trees (both scattered and single trees) (Adkins 2015). South Sudan has valuable tree species such as African blackwood (*Dalbergia melanoxylon* Guill. and Perr), African mahogany (*Khaya senegalensis* Juss.), teak (*Tectona grandis* L.f., introduced from South Asia), sapele (*Etandrophragma* sp.), *Isobertina doka* Craib and Stapf., the shea tree (*Vitellaria paradoxa* Gaertn), African cherry (*Prunus africana* (Hook.f.) Kalkman.), and the many acacia species, including the gum-producing *Acacia senegal* (L.) Willd. and *Acacia seyal* Delile to mention a few (MAF and RD 2012; Adkins 2015). The most important forest product is timber, which is obtained from both natural and plantation forests. In addition, non-forest timber products such as gums are of economic significance but currently not fully utilized. The forests are also an important source of firewood and charcoal, which account for over 80% of energy in South Sudan. Deforestation due to conflicts, population growth demand for fuel and charcoal and large-scale land acquisitions for mechanized farming is posing a serious threat to forest cover in South Sudan as well as elsewhere in SSA (Lawry et al. 2015). The expansion of cropland and loss of tree cover has dramatically increased and resulted in a decline in soil fertility and crop yields (Fahmi 2017). This situation thus invites new approaches to agriculture in the country.

2 AIMS OF THE RESEARCH

The main objective of this study was to bring new understanding about the effects of adding biochar alone or in combination with *Acacia seyal* agroforestry to the soil on growth and productivity of sorghum crop, and whether biochar addition would alleviate drought and water stress of sorghum crop under tropical semi-arid conditions.

Specific objectives were:

1. To determine whether the presence of *A. seyal* trees or addition of biochar or their interaction have effects on soil and subsequently on sorghum growth and yield; the trees in terms of facilitative or competitive effects and the biochar in terms of soil fertility and moisture conditions (**I**).
2. To determine whether biochar amendment can help to offset the effect of drought stress on sorghum biomass production and grain yield, and identify the morphological and physiological mechanisms involved (**II**).
3. To simulate the effects of biochar amendment on soil moisture conditions and sorghum biomass production and grain yield, and to determine whether the response differed between dry and wet years (**III**).

The corresponding research hypotheses of these three studies were:

1. The presence of leguminous *A. seyal* trees or addition of biochar significantly increases soil fertility and the availability and supply of water and thereby sorghum grain yields, and the effects of the trees and biochar on grain yields would be synergistic (**I**).
2. The effects of drought stress on sorghum leaf morphology (stomatal size, density) or physiology (conductance, transpiration, photosynthesis, leaf temperature and photosynthetic water-use efficiency) would significantly reduce sorghum grain yields, but the effects would be less when the soil is amended with biochar (**II**).
3. Reported increases in the water holding capacity of the soil due to biochar amendment would significantly increase simulated sorghum grain yields, and this effect would be greater in a drier year (**III**).

3 MATERIALS AND METHODS

3.1 General considerations

Detailed descriptions of the methods used are presented in the original publications (I, II, III). The present study consists of a field agroforestry experiment carried out in South Sudan (Study I), a greenhouse experiment using the same sorghum cultivar and seed carried out in Helsinki (Study II) but with light and temperature regimes mimicking those of the field experiment, and a crop biomass production and yield simulation using the AquaCrop model (Study III) parametrized for the field experiment site.

3.2 Agroforestry field experiment with biochar (I)

3.2.1 Field site

The agroforestry field experiment was setup at Magara Village (11° 58' 12" N, 32° 45' 36" E) north of Renk, in the Upper Nile state, South Sudan (Figure 1) and conducted during 2011-2012.

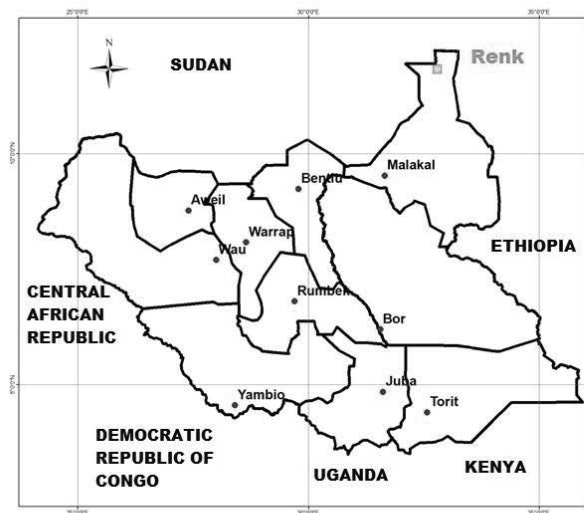


Figure 1. Map of South Sudan showing the location of the study area (Renk town).

The climate at the site is classified (Köppen-Geiger) as warm semi-arid (BSH) and the soil is a 30 cm thick silt loam layer underlain by clay. Two densities of *A. seyal* tree at the site were identified; scattered and dense, based on the distribution and arrangement of trees in the field. The experiment was set up in a split-plot design with three blocks and three treatment. Tree density was the main factor (plot size 10 x 10 m) and biochar amendment was the sub-plot factor (plot size 10 x 5 m). In summary, the experiment had three tree density treatments (Dense *A. seyal* + sorghum, Scattered *A. seyal* + sorghum, and sorghum only) and biochar treatment (0 or 10 t ha⁻¹). Pruning of lower branches of acacia trees and measurements of tree height, diameter at breast height, and canopy cover were carried out before sowing of sorghum seeds. Biochar was added to the soil before planting.

3.2.2 Biochar

The biochar used in this study was produced from *A. seyal* wood (commonly used for charcoal production in Sudan) using slow pyrolysis (350–500 °C) in traditional kilns (Schenkel et al. 1998). The biochar was crushed into more homogenous sizes and applied once at the start of the experiment (30/7/2011) and mixed into the 10 cm topsoil layer using hand hoes. The main characteristics of the biochar as well as scanning electron microscope (SEM) images are presented in Table 1 and Figure 2, respectively.

Table 1. Main properties of the *Acacia seyal* biochar used in the study (I).

Property	Value	Unit
pH (in water)	7.3	-
Liming efficacy compared to CaCO ₃	0.5	mol kg ⁻¹
Effective CEC	20.6	cmol(+) kg ⁻¹
Ash	24.0	%
Volatile matter	32.8	%
BET SSA	5.6	m ² g ⁻¹
C	69.4	%
N	0.08	%
C/N	86.8	-
Ca	22.9	g kg ⁻¹
Mg	13.5	g kg ⁻¹
K	95.8	g kg ⁻¹
P	16.8	g kg ⁻¹

n = 2 for volatile matter and liming effect, and n = 3 for the other analyses.

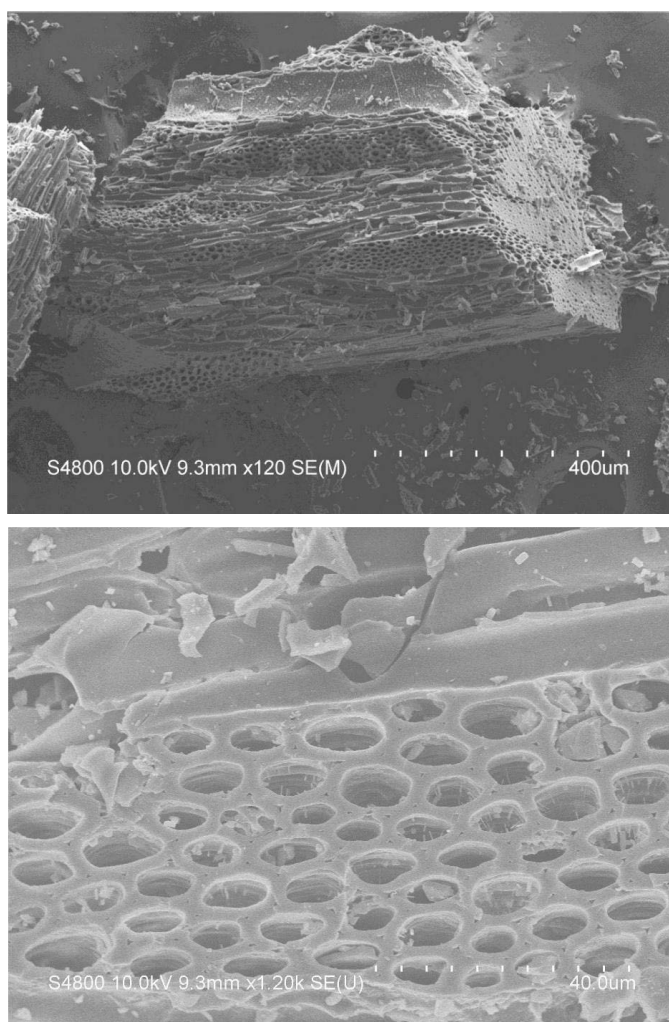


Figure 2. Scanning electron microscope (SEM) images of 0.2 mm sieved biochar particles. SEM images from the same sample were taken with 9.3 x 120 (top) and 9.3 x 1200 (bottom) magnification (study I).

Most of the biochar physiochemical properties were determined following methods similar to those used in the analyses of soil samples (Table 2). Volatile matter (VM) content was determined following the ASTM (2002) method where weight loss is recorded after heating at 910 ± 30 °C for 7 minutes. The liming effect was determined using 1 M HCl and titration with 0.1 M NaOH. Biochar specific surface area (SSA) was determined by the N₂ adsorption technique with a single point method (Tammeorg et al. 2014b). Scanning electron microscopy (SEM) images of biochar were produced using a Hitachi S-4800 field-emission scanning electron microscope. Elemental composition was determined using dry ashing followed by digestion in concentrated nitric acid and a microwave oven and determination of concentrations using inductively coupled plasma-optical emission spectrometry (ICP-OES).

3.2.3 Sampling and analyses

In Study I, soil samples of the 0–30 cm silty-loam layer were taken at the end of the second growing season. Soil analyses and methods used are outlined in Table 2.

In the field experiment, the height and stem diameter at breast height of the acacia trees were measured using a hypsometer and calipers at 1.3 m above the ground respectively. Crown cover (%) was calculated based on the crown area (ca) formula in equation 1 (Kuyah et al. 2012):

$$ca = \pi \left(\frac{l}{2} \right) \times \left(\frac{w}{2} \right) \quad (1)$$

where l is the crown diameter at its widest point and w is the perpendicular crown extent at the same height.

Prior to the start of the experiment, the number of stems of *A. seyal* trees was calculated per subplot and then upscaled for hectare-level values using the following formula (equation 2):

$$\text{Tree stems per ha.} = \text{No. trees per subplot} \times \left(\frac{10000 \text{ m}^2}{50 \text{ m}^2} \right) \quad (2)$$

3.3 Greenhouse water stress and biochar experiment (II)

The greenhouse experiment was conducted at the Viikki Campus (60° 13' 40" N, 25° 01' 03" E), Faculty of Agriculture and Forestry, University of Helsinki, Finland, during May–December 2011. The split-plot design was arranged with two experimental factors: drought stress (well-watered, medium drought and severe drought) and biochar addition (0 and 10 t ha⁻¹). The same Sudanese local variety of sorghum ('Wad Ahmed') as used in the field experiment (Paper I) was used and the soil mixture and greenhouse conditions were made so as to mimic the conditions at the site of the field experiment (Paper I). The biochar was brought over to Finland from Sudan and was produced from *A. seyal* using the same method as in the field experiment (Paper I).

3.4 Statistical analyses (I, II)

The statistical analyses were performed using SPSS software (SPSS Corp., Chicago, USA) package. Versions 22.0 and 23.0 were used for studies I and II, respectively. The effects of treatments were determined by univariate analysis of variance (ANOVA), and treatments were considered statistically significant at $p < 0.05$. Treatment means were compared using Tukey's HSD test and Student's t- test was used for testing the effect of biochar and the year (I).

3.5 Simulation of soil water availability and sorghum growth and yield response to biochar (III)

The simulation study (III) was carried out using the AquaCrop model (version 6.1) (Raes et al. 2018). Daily climate data (Climate Forecast System Reanalysis, CFRS) for a cell centred on the site was downloaded (<https://globalweather.tamu.edu/>) and the data for years 1990, 2011 and 2012 extracted. The year 1990 was the driest year amongst the data available (1979–2014), whereas the field experiment years 2011 and 2012 were the wettest ones. AquaCrop default soil hydraulic properties (saturation, field capacity, permanent wilting point and saturated conductivity) for silt loam and clay were used for the no biochar treatment and the effect of biochar on these properties was derived from the meta-analysis study by Omondi et al. (2016). The model was parameterized to severe soil stress so as to match the 2011 and 2012 field experiment sorghum yield data.

3.6 Summary of the methods used

The methods used in this study, including the field experiment, the greenhouse trial and the simulation exercise are summarized in Table 2.

Table 2. An overall summary of the methods used in this study, including the field experiment, the greenhouse trial and the simulation exercise.

Sample	Variable	Method	Reference	Publication
<i>Biochars</i>	pH	Standard combination electrode, 1:5 (w/w) in water		I
	Liming effect	Reaction with 1 M HCl and titration with 0.1 M NaOH		I
	Ash content	Dry combustion at 500 °C for 2 hours		I
	VM content	Weight loss after heating at 910 ± 30 °C for 7 minutes	ASTM (2002)	I
	Total C and N content	VarioMax elemental analyzer		I
	Total elemental composition	Treating ash with 0.2 M HCl, ICP-OES	Tammeorg et al. 2014b	I
	Specific surface area (SSA)	N ₂ adsorption technique	Tammeorg et al. 2014b	I
	Scanning electron microscopy (SEM) images	A Hitachi S-4800 Field-emission scanning electron microscope		I
<i>Soil</i>	pH	Standard combination electrode, 1:5 (w/w) in water		I
	Ash content	Dry combustion at 500 °C for 2 hours		I
	Total C and N content	VarioMax elemental analyzer		I
	Total elemental composition	HNO ₃ acid microwaved digestion using ICP-OES		I
	Exchangeable acidity	Titration of the 0.1 M BaCl ₂ extraction to a pH endpoint of 8.2 using 0.01 M NaOH		I
	CEC	Sum of 0.1 M BaCl ₂ extractable Ca, Mg, K and exchangeable acidity		I
	Particle size distribution	Laser diffraction device (Coulter LS230)		I
	Hydraulic properties (FC,PWP,AWC)	Calculated using measured clay, silt, sand and organic matter contents and pedotransfer functions (Saxton and Rawls 2006)		I
<i>A. seyal</i>	Stem	Calculated (No. of trees per subplotx10000/50)		I
	Diameter at Breast Height (dbh)	Calipers at 1.3 m above the ground		I
	Height	Hypsometer		I
	Canopy cover	Calculated (crown area/50x100)	Kuyah et al. 2012	I

Sample	Variable	Method	Reference	Publication
<i>Sorghum growth</i>				
	Plant height	Scaled ruler		I–II
	Plant stem diameter	Vernier calipers		I–II
	Panicle length	Ruler		I–II
	No. of leaves per plant	Counting		I–II
	No. of seeds per plant	Counting		I–II
<i>Sorghum stomata and physiology</i>				
	Net photosynthesis	LI-6400 portable open gas exchange system (average of four measuring occasions)		II
	Transpiration rate	LI-6400 portable open gas exchange system (average of four measuring occasions)		II
	Leaf temperature	LI-6400 portable open gas exchange system (average of four measuring occasions)		II
	Water use efficiency	Photosynthesis rate/transpiration %		II
	Stomatal conductance	LI-6400 portable open gas exchange system (average of two measuring occasions)		II
	Stomatal density	Calculated (average stomatal count divided by the area of the field of view)		II
	Stomatal length	Laborlux S binocular microscope	Voleníková and Tichá, 2001	II
	Stomatal width	Laborlux S binocular microscope		II
	Number of stomata	Counted from three fields of view on each adaxial and abaxial imprint		II
<i>Sorghum grain yield (dry weight)</i>				
	Grain yield (t ha ⁻¹)	Calculated ten plants sample per subplot, and then upscaled per hectare		I
	Grain yield (g plant ⁻¹)	Calculated per plant		II
	Simulated grain yield (t ha ⁻¹)	AquaCrop model (v. 6.1)	Raes et al. 2018	III
<i>Sorghum biomass (dry weight)</i>				
	Total biomass (t ha ⁻¹)	Above ground biomass (panicle and shoots) + root biomass		I–II
	Biomass (t ha ⁻¹)	Sun drying to constant weight		I
	Biomass (t ha ⁻¹)	AquaCrop model		III
	Biomass (g plant ⁻¹)	Oven dried at 80 °C for 24 h using a Memmert162 dryer		II

4 RESULTS AND DISCUSSION

4.1 Effect of acacia-based agroforestry on soil

The *A. seyal* trees had significantly increased the C/N ratio of the soil, total and exchangeable Ca^{2+} under scattered *A. seyal* treatment, but lowered the pH, N content compared to sole sorghum treatment. However, the trees did not have significant effects on other tested soil properties such as soil carbon, total and exchangeable Mg^{2+} , CEC as well as hydraulic properties (Table 1, Paper I). Due to inputs of *A. seyal* litterfall to the soil and its slow rate of decomposition (Bernhard-Reversat 2002; Ahmed et al. 2018), soil organic matter was expected to increase. This would account for the observed increase in soil C and nutrient contents, CEC and water retention in the acacia trees plots. Such increases have been reported in other agroforestry studies on drylands. For instance, El-Tahir et al. (2004) found an increase in P, N and C contents under *A. seyal* on sandy soils in North Kordofan compared to bare soil and to the soil under *A. senegal* or *A. tortilis*. Furthermore, several authors have reported higher contents of organic matter, N, P, K and Ca under dryland tree canopies compared to open lands (Belsky et al. 1989; Buresh and Tian 1998; Abdallah and Chaieb 2012; Desta et al. 2017).

In the present study, the significantly higher soil N content observed under sole sorghum compared to *A. seyal* intercropping was unexpected, because of the assumed N fixation by the acacia trees (Buresh and Tian 1998; Ahmed et al. 2018). It maybe that N fixed by the acacias was offset through mechanisms, such as mineralization, and subsequent loss through uptake by the trees or leaching resulting in soil organic matter with lower N contents. Therefore, the differences in soil C/N ratios among the cropping systems could be due to variation in the N content rather than that of the C content of the soil organic matter. The lower soil pH associated with acacia intercropping compared to sole sorghum, which was significant in the case of scattered acacia trees, may be attributed to the relatively high concentration of phenolic compounds found in acacia litter, particularly *A. seyal* (Bernhard-Reversat 1987).

4.2 Effects of biochar on soil quality

4.2.1 Biochar in acacia agroforestry (I)

The application of biochar in the field experiment (with or without acacia trees, I) increased the contents of C and exchangeable K^{+} and improved the soil hydraulic properties as indicated by higher FC and plant AWC, but decreased the content of both exchangeable Ca^{2+} and CEC (Paper I, Table 3). In contrast, biochar appeared to have no significant effects on the soil pH and the total mineral nutrient and sulphur contents. Although the content of exchangeable K^{+} increased in the soil as a result of the biochar, it was offset by reductions in the contents of exchangeable Ca^{2+} and Mg^{2+} , which in turn reduced the values of CEC. The increase in the soil K content after addition of biochar may have been due to a fertilizer effect, as the woody biochars are rich in K (Glaser et al. 2002; Tammeorg et al. 2014a, b).

Previous studies on the effects of biochar on soil properties have demonstrated that it can increase nutrient retention in some soils, particularly in highly weathered, degraded, acidic and sandy soils with low initial carbon contents (Cornelissen et al. 2013; Jien and Wang 2013; Liu et al. 2013; Nelissen et al. 2015; Laghari et al. 2015; Omondi et al. 2016; Atkinson 2018; Günal et al. 2018). There is also mounting evidence that biochar can also initiate an improvement in some physicochemical properties of clayey soils (De Melo Carvalho et al. 2013; Ouyang et al. 2013; Tammeorg et al. 2014a; Günal et al. 2018; Rasa et al. 2018). However, Pituello et al. (2018) have reported in their recent meta-analysis study that the application of biochar in large amounts (40 t ha^{-1}) changes the chemical properties but weakens the stability of clay aggregates.

Many biochars studies from the tropics (Liang et al. 2006; Major et al. 2010a; Peng et al. 2011) have also observed an increase in soil Ca contents related to biochar application. The reduction in soil Ca^{2+} and Mg^{2+} contents in this study could have been a result of exchange with K^{+} and subsequent leaching (Tammeorg et al. 2014c). In addition to the reduction in exchangeable Ca^{2+} and Mg^{2+} , the reduction in CEC could have been a dilution effect, as the CEC of the biochars was half that of the untreated soil and thus the addition of the biochar would have lowered soil CEC.

The application of biochar had no significant effect on soil pH, which is a logical result as the woody biochar had a low liming capacity. Furthermore, the soil pH at the field site was nearly alkaline, which was similar to that pH of the biochar used (Paper I, Tables 2 and 3). Although the N contents were higher in the biochar-treated soil as compared to that without biochar, the difference was not statistically significant. These findings are in contrast to other studies that have found biochar to increase soil N contents under intercropping with N-fixing plants, primarily due to improved availability of B and Mo (Rondon et al. 2007; Van Zwieten et al. 2015).

4.2.2 Simulated effects of biochar on rooting zone soil water contents (III)

The AquaCrop-simulated root zone soil water contents were close to permanent wilting point throughout 1990 but remained at or above field capacity during the first half of the growing season in 2011 and 2012. High simulated soil water contents, reaching saturation on some days, were associated with very heavy rainfall events. However, contents declined towards permanent wilting point during the second half of the 2011 and 2012 growing season.

Based on the meta-analysis study by Omondi et al. (2016), biochar amendment increases the water holding capacity of the soil and therefore the supply of water to plants. The degree of increase in soil water retention varies with biochar dose and soil texture type. Moreover, the percentage increases in hydraulic properties used in the simulations (7% in θ_{SAT} , 10 and 20% increases in θ_{FC} , and 20% and 27% increases in K_{sat}) represent the range in changes for medium textured soils (loam-silt) which include silt loam soil, reported by Omondi et al. 2016 and therefore are appropriate for the field experiment site. It was assumed in this study that the biochar-induced increases in AWC were caused by an increase in FC. According to Omondi et al. (2016), some studies attribute the increase in soil porosity to increases in small and medium sized pores in the soil while others have attributed it to the microporosity of the biochar itself.

Given the increases in soil hydraulic properties that could realistically be achieved by adding biochar to the soil at the field experiment site, biochar only increased the soil water content of the rooting zone in 1990. This finding confirmed the hypothesis that biochar-related improvements in soil hydraulic properties only increase sorghum yields in very dry years (1990). Nevertheless, the overall difference in daily simulated soil water contents of the rooting zone between the biochar scenarios and that without biochar was small. Thus, the absolute differences in daily-simulated water contents of the rooting zone for biochar treatments varied between 3 and 9 mm across all three years. These changes occurred mostly in the beginning of the growing season and corresponded to only 3–6% increase in the rooting zone soil water content compared to soils without biochar.

4.3 Sorghum yields in acacia-based agroforestry

A. seyal intercropping negatively affected sorghum growth and yield (Paper I, Figure 4 A). Sorghum grain yields without trees were 400 % and 300% higher than yields in dense acacia intercropping for 2011 and 2012, respectively, and yields in 2011 were only half of those in 2012.

Land Equivalent Ratios (LER) for sorghum intercropped with *A. seyal* only slightly varied as a result of biochar addition compared to those for sole sorghum. The LER value of 0.3 for the dense *A. seyal* intercropping in 2011 and 2012 was the same as for the scattered *A. seyal* intercropping in 2011 but a double (0.6) in 2012. Nevertheless, the LER were < 1.0, indicating that *A. seyal* trees exhibited a clear negative (i.e. competitive) effect on sorghum crop.

This finding is in agreement with those of Gaafar et al. (2006), who observed a significant decrease in sorghum and karkadeh (*Hibiscus sabdariffa*) yields when grown under young (6-year-old) *A. senegal* trees in North Kordofan, Sudan. The authors attributed the yield reduction to competition between the tree and crop roots for water. Similarly, Raddad (2006) has reported a reduction in sorghum grain yield when intercropped with young *A. senegal* trees on clayey soil in the neighbouring Damazin area across the border in Sudan. The reduction in sorghum yield was again attributed to competition between trees and sorghum plants. Fadl and El Sheikh (2010) observed a significant reduction in the yields of groundnut (*Arachis hypogaea*), karkadeh (*Hibiscus sabdariffa*) and sesame (*Sesamum indicum*) intercropped with relatively old (15-year-old) *A. senegal* trees in North Kordofan and came to the same conclusions as for explaining factors.

In contrast, a number of studies have reported positive effects (facilitation) of acacia trees intercropped with sorghum and other crops (Raddad et al. 2006; Fadl 2013; Ahmed et al. 2018). These positive effects were attributed to increased soil fertility as a result of the acacia trees and increase in soil organic matter (Ahmed et al. 2018). Abdoukadi et al. (2019) reported that although the growth and yield of pearl millet (*Pennisetum glaucum* (L.) R.Br. in Niger were higher out of the canopy than under the canopy of *A. senegal*, averaged across the whole parkland system, growth and yields were higher compared to pure millet culture. These contrasting results on crop yields mixed with trees suggest that the effect of trees depends on factors related to tree species, age, size and density, as well as on the annual variation in climatic conditions, especially, precipitation (Danso et al. 1992). For example, Fahmi (2017) has reported, from Sinnar state, Sudan, across the border from the present field study site, positive effects of acacia parkland agroforestry on clayey soils on crop yields; these studies included sorghum, pearl millet and sesame as agricultural crops. The author attributed the increase in yields to the fact that the acacia trees (*A. seyal*, *A. senegal* and *A. mellifera*) were naturally regenerated, mature (old) and scattered. Fahmi et al. (2018), in a later paper, concluded that well-established, old acacia trees do not compete heavily with crops for water or soil nutrients compared to the situation with young acacia trees, as in our present study (I). Fahmi (2017) also carried out economic analyses of the cropping systems and found that parkland agroforestry, apart from a crop yield increase, also may result in substantial financial benefits to smallholder households compared to monocropping.

Since *A. seyal* trees are known to have long taproots (typically around 120 cm) and fine lateral roots (Adams 1967), it was expected that acacias in the present study would meet their water requirements from deeper soil layers rather than competing with their understory sorghum plants (Adams 1967; Gaafar et al. 2006). However, it may be that the acacia roots in our field experiment were not yet well established deeper in the soil, resulting in competition with sorghum roots for resources in the topsoil. The restriction in rooting depth may be related to the young age and relatively small size of acacia trees or caused by the underlying compact clay deposit (Bukhari 1998). Thirdly, *A. seyal* has a denser canopy cover than, for instance, *A. senegal*, and sorghum is known to grow poorly in shade (Wilson et al. 1998; Gnangle et al. 2013). Therefore, the observed decline in sorghum yield in *A. seyal* intercropping could also be the result of shading (Belsky 1994).

4.4 Effect of biochar on sorghum yields

4.4.1 Biochar and sorghum yields under field conditions (I)

The expectation in this study was that addition of biochar would increase sorghum yields due to improved soil moisture and retention capacity. For example, Cornelissen et al. 2018 have reported that application of coco shell biochar (15 t ha⁻¹) increased maize yield in an acidic sandy loamy soil, Indonesia. These biochar positive effects were attributed to alleviation of soil acidity and nutrient availability. Danso et al. (2019) have recently reported an increase in maize yield after application of rice straw biochar at higher rate 30 t ha⁻¹ to a sandy clay loam soil, Ghana. This yield increase was attributed to increase in light interception by maize plants, which was in turn a result of improved soil moisture and nutrient retention. Similarly, Kamau et al. (2019) have reported an increase in maize yield in biochar amended nitisols, Kenya. The authors have attributed this increase to increase in P content.

However, and in contrast to the effect of the acacia trees, addition of biochar had no significant effect on sorghum grain yield (Paper I, Figure 4 B). This could be attributed to either biochar not affecting the pH of the alkaline soil, and/or having a limited fertilizing effect which was not enough to support biochar's induced improvement in soil water storage and lead to crop yield improvement (Lentz and Ippolito 2012; Scott et al. 2014). This result is consistent with the findings of De Melo Carvalho et al. (2013), who reported that an addition of biochar to a clayey soil in the Brazilian savanna did not have any significant effect on rice grain yields. Cobb et al. (2018) have also reported from their pot experiment in the USA that an addition of pinewood biochar alone to sandy soil did not significantly increase sorghum yield compared to unamended control.

It has previously been reported that biochar has variable effects on crop yields depending on biochar feedstocks, applied doses and time since application, soil type and prevailing climate (Blackwell et al. 2009; Major et al. 2010b; Biederman and Harpole 2013; Liu et al. 2013; Kamau et al. 2019). Cornelissen et al. (2013) observed that yield responses to biochar varied with soil type (acidic and neutral clay-loams and silty clay) in Zambia. Hairani et al. (2016) reported a 1.5-fold increase in sorghum plant height after the application of

rapeseed cake biochar to a Gleyic fluvisol in pot experiments in Japan. The authors attributed this growth improvement to biochar-induced changes in the microbial community structure in soil.

4.4.2 Biochar effect on sorghum yields under greenhouse conditions (II)

Similarly, to the results on sorghum yields in the field experiment (I), a biochar addition in the greenhouse study (II), did not show any statistically significant effects on sorghum. In the greenhouse experiment, apart from grain and biomass yields, various physiological characteristics related to gas exchange were also studied (Paper II, Table 4).

The reported benefits from soil treatment with biochars for crop growth and yield under water-limited conditions are linked to improvement in soil water holding capacity (Kammann et al. 2011; de Sousa Lima et al. 2018). The increase in soil carbon as a result of biochar addition under greenhouse conditions in the present study was expected to increase the soil AWC, similarly to the significant increase in SOC found in the field study (I). As mentioned earlier, the effect of biochars on soil water retention depends on characteristics of both soil and biochars (Ding et al. 2016; Tammgeorg et al. 2017; El-Naggar et al. 2019); however, the effect is largely determined by the initial SOC content. This is because the SOM content is a key determinant of the surface properties of the soil and sorption of water molecules (Murphy 2015), thus allowing the polar functional groups on the biochars surface to serve as water adsorption cores and facilitate the formation of water clusters on the surface (Cybulak et al. 2016).

Consequently, it was assumed in this work that an addition of biochar at a higher rate than 10 t ha^{-1} would raise the SOC content to more than 5% and thus induce significant changes in the soil AWC. This was consistent with the findings of Keshavarz Afshar et al. (2015), who reported that an addition of 2% biochars to soil having an initial C content of 41 g kg^{-1} did not induce significant effects on AWC. Surprisingly enough, in our field experiment (I), the AWC values were similar both with and without biochar; therefore, any possible effect of biochar had to be a result of changes in such physiological characteristics as stomatal morphology or gas exchange. In contrast, Kammann et al. (2011) observed that application of biochar derived from peanut hull feedstocks at high doses (100 and 200 t ha^{-1}) not only improved the growth of *Chenopodium quinoa* Willd. plants in sandy soil but also increased their leaf N, drought tolerance and WUE. This was attributed to increases in K^+ ions, changes in osmotic activity and stimulated fine root growth.

In the field study (I), an addition of the same *A. seyal*-derived biochar at the same rates as in the greenhouse experiment study (II), biochar increased the soil K^+ contents by 32% and the total K content was 96 g kg^{-1} . Although a severe drought stress in the greenhouse study reduced the root biomass by 42% compared to the situation for well-watered plants, the biochars addition had no effect on root biomass (Paper II, Table 4).

Another possible explanation for the effect of biochars under water stress could be linked to phytohormonal signalling (Kammann and Graber 2015). For instance, Di Lonardo et al. (2013) have attributed improved root development of white poplar clones to the adsorption of growth-limiting phytohormonal ethylene by biochars added to a soilless agar growth medium. However, application of *A. seyal* biochar corresponding to 10 t ha^{-1} in the current greenhouse study did not have significant effect on any measured variable in sorghum (stomata morphology, gas exchange, or biomass or grain yields).

For comparison, Keshavarz Afshar et al. (2015) reported that an application of maple wood biochars at doses of 1% and 2%, or their interactions with drought stress under greenhouse conditions, did not show any significant effect on the physiological traits measured (photosynthesis and transpiration rates, and stomatal conductance) in milk thistle (*Silybum marianum* L. (Gaertn.) plants. The authors explained this lack of response to the initially high C content of the soil coupled with an application of biochars at low doses, and to the duration of the experiment that was not long enough to cause a significant impact on soil properties or plant performance under drought stress. The above-mentioned positive effects observed in a greenhouse experiment with *Chenopodium quinoa* by Kammann et al. (2011) were attained in sandy soil and with higher biochars application rates (100 and 200 t ha^{-1}) than those used in the present study. Therefore, it can be inferred that ecophysiological effects of biochars on crops grown under drought stress could be demonstrated only with high application doses and limited to conditions of sandy soils and a long observation period (Cornelissen et al. 2013; Mulcahy et al. 2013).

4.4.3 Simulated biochar effects on soil moisture availability and Sorghum yield (III)

In the present study, there was a clear difference between the AquaCrop simulated sorghum biomass and grain yield in a dry (1990) year and wet years (2011 and 2012) (Paper III, Figure 3). Based on average values of biochar additions, the biomass production in 1990 was 67% of that in 2011 and 71% of that in 2012. On the other hand, the grain yield was less affected, and the grain yield production in 1990 was 72% and 81% of the yields in 2011 and 2012, respectively. The effect of biochar amendment on biomass and grain yields was only noticeable in 1990. Furthermore, the increase in both biomass and grain yield in 1990 was 33% with the two highest levels of biochar additions compared to the yield without biochar, while the increase was only 14% with the two lowest levels.

In the wet years 2011 and 2012 the effects of biochar on biomass and grain yields were small, only showing a 2% increase. The higher biomass and grain yields observed under the two highest biochar scenarios were considered attributable to increases in soil water storage capacity (θ_{fc}) rather than changes in permeability. Although the different added biochar scenarios affected the simulated soil water content of the rooting zone when compared to no biochar addition, the increase was clearer in 1990 when it caused a distinct increase in biomass and grain yields compared to the wet years (2011 and 2012). This is in agreement with the hypothesis made in this study that biochars have greater effects on biomass and grain yield in dry years.

Moreover, the simulated and measured biomass and grain yields were generally low, especially in the driest year of 1990 (Paper III, Figure 3). The results also show that an addition of biochar increases the soil water storage (TAW), but without improvement in biomass or grain yield. Because sorghum in dryland Africa is largely grown by smallholder farmers under rainfed conditions and on marginal land with low input practices and using local landraces, its yields are typically low, 0.5-0.9 t ha⁻¹ (Steduto et al. 2012).

Sorghum yield studies from Sudan have shown that there is not only large disparity in sorghum grain yield among different varieties and cultivars but also within the same variety under different climatic settings. For example, the ‘Wad Ahmed’ cultivar used in the present study is an officially released and standardized sorghum variety in Sudan that is used as reference in many studies, but it has also been reported to perform poorly under some conditions (Bahar et al. 2015).

4.5 Sorghum response to water stress

4.5.1 Plant stomata morphology and gas exchange (II)

Drought stress significantly affected the gas exchange characteristics in sorghum (stomatal conductance, photosynthesis, transpiration, and photosynthetic WUE), especially under severe water deficit, but it did not influence the stomatal morphology (stomatal size and density for abaxial or adaxial leaf surfaces; Paper II, Tables 1-3). For instance, the stomatal conductance was significantly reduced under severe drought compared to the situations with medium drought and well-watered plants. A similar pattern was observed for photosynthesis and transpiration rates, which also were significantly reduced under severe drought (Paper II, Figures 7A-C).

Although the photosynthetic WUE was observed to increase proportionally to the level of drought stress, significant differences in this parameter were only recorded between plants subjected to severe drought and those with no water stress (Paper II, Figure 7D). The amount of reduction in sorghum gas exchange due to imposed drought was 56%, 18% and 31% in stomatal conductance, photosynthesis, and transpiration, respectively, under severe drought stress when compared to the well-watered treatment (Paper II, Figure 7E).

Stomatal conductance is defined as a function of stomatal density, degree of stomatal aperture and stomatal size, and it controls a plant's gas exchange parameters such as photosynthesis (CO₂ uptake) and transpiration (water loss) (Larcher 1983). Earlier research has shown that developing plants show a decrease in stomatal size and an increase in stomatal density when subjected at least to moderate drought stress (Muchow and Sinclair 1989; Xu and Zhou 2008). Recently, the role of a peptide signaling mechanism in epidermal cells of plants has been reported by Hepworth et al. (2015); it regulates the formation and density of stomata at the time of leaf formation.

Although the values for stomatal size and density for sorghum in this study (II) are in agreement with those reported by Turner and Begg (1973), and Muchow and Sinclair (1989), drought stress did not induce significant

variation in these parameters (Paper II, Table I). According to the reported variation in stomatal size and density among sorghum genotypes (Muchow and Sinclair 1989), different responses of a plant's stomatal size and density to drought stress can be expected during the growth and development of the plant in question. As reported in earlier work, the significant differences in photosynthesis, transpiration and WUE now observed between well-watered plants and those under severe drought stress could be a result from variation in stomatal opening control (Chaves 1991; Mastrorilli et al. 1995; Massacci et al. 1996). This would be in agreement with results of Fracasso et al. (2016), who found that the stomatal conductance, photosynthetic rate, and transpiration rate in all sorghum genotypes tested were significantly different between well-watered plants and those under water stress. A reduction in CO₂ assimilation in plants in reaction to drought is mainly mediated by a decrease in stomatal conductance (cf. Farooq et al. 2009; Fracasso et al. 2016); however, especially under severe water stress conditions, contributions of non-stomatal biochemical factors cannot be excluded (Ghannoum 2009; Keshavarz Afshar et al. 2015).

4.5.2 Crop biomass and grain yield

4.5.2.1 Effects of water stress under greenhouse conditions (II)

Under greenhouse conditions (II), the effects of drought stress on sorghum biomass and grain yields were consistent, both parameters showing decreasing trends with an increasing level of drought stress (Paper II, Table 5). The greatest reduction was associated with the number and weight of seeds produced per plant. Compared to well-watered plants, those exposed to severe drought showed a 92% and 95% reduction in the number and weight of seeds produced per plant, respectively.

It is apparent from the vast literature on sorghum cultivation that subjecting the plants to drought stress hinders their development and reduces biomass and grain yields (Ahmed et al. 2011; Menezes et al. 2015; Khaton et al. 2016; Batista et al. 2019). The effect can be severe during both the vegetative and the reproductive stage of development (Craufurd 1993; de Oliveira Neto et al. 2014). In the present study (II), drought negatively affected the sorghum biomass and grain yields despite an increase in WUE found with increasing water stress.

These results are in agreement with the established understanding that the sorghum plant is relatively more sensitive to drought during the reproductive stage than many other crops, and that a stress during this stage decreases both the seed number and seed size and thus leads to lower yield (Manjarrez-Sandoval et al. 1989; Beheshti and Behboodi Fard 2010; Tsuji et al. 2003; de Oliveira Neto et al. 2014; Hmielowski 2017; Batista et al. 2019).

4.5.2.2 Sorghum yields as related to climatic factors (crop simulation study, III)

In the present study, the amount of annual rainfall for 1990 was 207 mm; this was about a fifth of that in 2011 (836 mm) or 2012 (899 mm). The higher rainfall in 2011 and 2012 was mainly the result of a few days of very heavy rainfall incidents (Paper III, Figure 1). The maximum daily rainfall was 21 mm in 1990, 146 mm in 2011 and 124 mm in 2012. In addition, the number of rainy days was 92 days in 1990, 111 days in 2011 and 109 days in 2012.

The pattern of rainfall distribution varied noticeably between 2011 and 2012, with the most rainfall concentrated in the beginning of the season in 2011. However, the situation was different in 2012 when the rainfall also occurred before the growing season and was marked with even distribution over the season. This had a clear effect on the soil water content during the growing seasons of the two years. Most of the daily rainfall infiltrated into the soil, but a considerable surface runoff was observed both in 2011 and in 2012. Reflecting the distribution in the heavy rainfall days, much of the surface runoff in 2011 occurred during the growing season, while in 2012 most of it occurred before the growing season. Daily ET, as could be expected, to some extent mimicked the rainfall distribution.

In comparison to maize and some other cereal crops, sorghum is known to be more drought-tolerant but showing a distinct response to water stress at various growth stages that is reflected in respective variations in the transpiration rate and WUE (Pinheiro and Chaves 2011). However, in the present study, a clear positive correlation was observed between the annual rainfall and simulated sorghum yields for all three years studied

(Paper III, Figure 3). Msongaleli et al. (2017) have similarly observed an impact of rainfall variability on sorghum grain yields from their APSIM model study in semi-arid Tanzania.

Sorghum grain yields now observed in the field (I) and in AquaCrop simulation (III) were in both cases inconsistent with those reported by Msongaleli et al. (2017), as the grain yield in our study in 2012 was greater than that in 2011, with opposite results obtained from AquaCrop simulation. The relatively high grain yields observed in the field in 2012 compared to those in 2011 (I) could be attributed to a more even distribution of rainfall and, consequently, of the soil water content in the rooting zone during the 2012 growing season. On the other hand, the higher AquaCrop simulation grain yields in 2011, compared to those in 2012, could be due to the effect of the relatively wet first half of the growing season in 2011. It can thus be concluded that the growth, yield and phenology in sorghum is a result of a complex combination of effects of both the amount and temporal distribution of rainfall.

5 CONCLUSIONS

This study has produced information about the effect of trees and biochar amendment on sorghum production in semi-arid South Sudan.

The results of the *Acacia seyal* agroforestry experiment highlighted the complexity of rainfed crop production in the area. It was expected that the inclusion of trees and the biochar amendment would result in increased sorghum biomass production and, more importantly, grain yields. However, sorghum biomass and grain yields were found to be less in the presence of trees. This negative effect of the trees on sorghum production would indicate that, contrary to the propounded benefits of agroforestry, the *A. seyal* trees were in competition with the sorghum crop for soil water and nutrients and/or that the shading by the trees was detrimental to sorghum growth. The competition for water and nutrients may have been because there was no separation in rooting depth between the trees and sorghum plants. Unfortunately, no information on rooting depths was taken during the course of the experiment. However, as sorghum is not tolerant of shade, it can be concluded that the reduction in sorghum production with increasing tree density was probably due to canopy cover and shading.

Sorghum is known to be drought tolerant. As shown by the greenhouse experiment, drought stress has no effect on sorghum stomatal traits, but does on gas exchange, and sorghum growth and grain yields decrease with the degree of drought stress. The greenhouse experiment results indicate that the drought tolerance of sorghum is related to ecophysiological responses rather than to morphological responses. The results also showed that sorghum plants can tolerate and survive considerable levels of drought stress.

Biochar application was found to improve some soil physicochemical properties. The field experiment showed that biochar significantly increased soil C, exchangeable K^+ contents and soil hydraulic properties (field capacity and available water capacity). However, contents of exchangeable Ca^{2+} and cation exchange capacity were reduced and there was no effect on soil pH. Nevertheless, in spite of the positive changes in soil properties, biochar did not increase sorghum growth and yield as expected. Similarly, the biochar amendment in the controlled conditions of the greenhouse experiment did not increase the biomass production or grain yield of sorghum, and the results from AquaCrop growth simulation study indicated that increases in soil hydraulic properties resulting from biochar amendment only increase sorghum biomass production and grain yields in very dry years. Past literature suggests that more time or higher doses of biochar may be required before benefits in crop production can be observed. Therefore, long-term studies may be needed in order to fully assess whether adding biochar to the soil in semi-arid environments can improve crop production.

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